CARBON USE BY THE ENDANGERED TEXAS WILD RICE
(*ZIZANIA TEXANA*, POACEAE)

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ABSTRACT

A pH drift experiment was conducted to determine the HCO_3^- uptake potential of Texas wild rice (*Zizania texana* Hitchc.). In 15 separate experimental runs, total inorganic carbon to alkalinity ratio varied between 0.93–0.99 and final pH varied between 8.54–9.21, strongly suggesting Texas wild rice is an obligate CO_2 plant unable to utilize HCO_3^-. The estimated CO_2 compensation point for Texas wild rice is 2 µmol l^{-1}.

Data from this study provide physiological evidence for the positive relationship between current velocity and biomass productivity observed in Texas wild rice (Power 1996a, 1996b, 2000; Poole & Bowles 1999; Saunders et al. 2001) and provides water resource managers with information they need to manage and protect the San Marcos River ecosystem.

RESUMEN

Un experimento de la variación del pH fue utilizado para determinar el potencial de absorción de HCO_3^- del arroz salvaje de Tejas (*Zizania texana* Hitchc.). En 15 experimentos separados, la relación entre el carbono inorgánico y la alcalinidad varió entre 0.93–0.99 y el pH final varió entre 8.54–9.21, sugiriendo que el arroz salvaje de Tejas es una planta CO_2 obligatoria incapaz de utilizar el HCO_3^-. El punto de compensación del CO_2 en el arroz salvaje de Tejas es 2 µmol l^{-1}.

Los datos de este estudio proporcionan la evidencia fisiológica para la relación positiva entre la velocidad actual y la producción de la biomasa observadas en el arroz salvaje de Tejas (Power 1996a, 1996b, 2002; Poole y Bowles 1999; Saunders et al. 2001) y ofrecen a los encargados de los recursos hídricos la información que necesitan manejar y proteger el ecosistema del río San Marcos.

INTRODUCTION

The San Marcos River arises from springs within the City of San Marcos, Hays County, Texas, and flows 8.1 river km in a southeasterly direction before it joins the Blanco River. The source aquifer for the San Marcos River is the Edwards Aquifer, the sole source of municipal, agricultural, and industrial water for 1.5 million people in central Texas. Water is pumped continuously from the Edwards Aquifer for human use, but is recharged only during rain events. As water is pumped during drought periods when recharge is minimal, the level of the aquifer drops, threatening spring flow to the San Marcos River and other local spring systems. The spring-fed San Marcos River provides habitat for five federally listed species: fountain darter (*Etheostoma fonticola*), San Marcos gambusia (*Gambusia georgei*), San Marcos salamander (*Eurycea nana*), Texas

blind salamander (*Typhlomolge rathbuni*), and Texas wild rice (*Zizania texana*). The primary threat to each species is reduced spring flow from the source aquifer (U.S. Fish and Wildlife Service 1996). The threat became critical during a drought event in 1990 and all five species were the focus of an endangered species lawsuit initiated in 1991 (Sierra Club v Lujan, No. MO-91-CA-69). One outcome of the lawsuit was creation of a water authority to manage the aquifer for human use and to protect the San Marcos River ecosystem for endangered species by maintaining adequate spring flow. Defining "adequate" spring flow for a species is a monumental challenge met by research systematically addressing ecological and physiological aspects of the species.

The target species for this study was Texas wild rice. This species is endemic to the San Marcos River, TX and was listed as endangered in 1978 (U.S. Fish and Wildlife Service 1985). Texas wild rice is a perennial, submersed macrophyte now commonly found in swiftly flowing water. Under these conditions, it produces long, ribbon-like, submersed leaves and reproductive culms. Culms can become emergent and produce wind pollinated, terminal inflorescences. Nodes along each culm give rise to roots and asexual clones, called tillers. A thorough description of the species and its habitat is provided by Terrell et al. (1978), U.S. Fish and Wildlife Service (1995), Poole and Bowles (1999), and Saunders et al. (2001).

Texas wild rice has two distinct phenotypes under wild and cultured conditions. Wild plants found in flowing water (>0.46 m s^{-1}) in the San Marcos River are perennial and primarily submersed, although reproductive culms are present. Under cultivated conditions in slow moving water (<0.05 m s^{-1}), Texas wild rice has low vegetative productivity compared with study plants grown in faster flowing water ranging from 0.29-0.49 m s^{-1} (Power 1996a, 2002). Texas wild rice in slow moving water ranging from 0.001-0.12 m s^{-1} is primarily emergent, sexually reproductive, and annual to short-lived as is common for other North American *Zizania* species (Terrell et al. 1978; Power 1996a, 2002). Primarily emergent plants with low biomass productivity are especially vulnerable to herbivory because of the potential for loss of reproductive culms prior to seed set and the ultimate loss of the plant because of a shortened life span.

Carbon utilization by macrophytes has been shown to influence photosynthesis in experimental studies (Smith & Walker 1980) and may be an important factor influencing phenotypic variation in Texas wild rice. The relative concentrations of carbon species dissolved in water are influenced by pH. At lower pH proportionally more CO_2 is available; as pH rises, equilibrium shifts to HCO_3^-, then CO_3^-. Macrophytes utilize inorganic carbon in the form of CO_2 for photosynthesis and all submersed species are able to extract this form of dissolved inorganic carbon from the water when it is available. In addition, some submersed species show the ability to extract efficiently HCO_3^- from the water while others exhibit little or no ability to do so (Allen & Spence 1981; Maberly

& Spence 1983; Sand-Jensen 1983; Spence & Maberly 1985). In addition to the absolute concentration of dissolved inorganic carbon, the flow environment of the submersed leaves also influences their ability to extract carbon from the water. Flowing water reduces the thickness of the boundary layer surrounding leaves through which gases move predominantly by diffusion. Slower diffusion of CO_2 across the boundary layer can limit photosynthesis in still or very slow moving water (Smith & Walker 1980).

Differential carbon availability combined with carbon uptake potential may contribute to phenotypic variation in Texas wild rice and provide a physiological explanation for its preference for a flowing water habitat. The purpose of this study was to determine the bicarbonate uptake potential of Texas wild rice.

METHODS AND MATERIALS

Carbon use by Texas wild rice was examined using plants taken from culture after seedlings had been growing about three months in an outdoor cement raceway. The raceway was supplied with water pumped from the Edwards Aquifer and with chemistry similar to that of the San Marcos River. The plants had 4–6 leaves, each of which was 20–30 cm in length. The plants were maintained in aerated river water (4.5 meq l[-1] alkalinity) under artificial illumination (250 $\mu mol\ m^{-2}\ s^{-1}$ PAR; 14:10 light/dark photoperiod) at laboratory temperature (22° C) until utilized. All experiments were conducted within four days of arrival of plant material at the lab.

For comparison data are also presented for *Hydrilla verticillata*, a submersed aquatic species known to be well adapted for extracting bicarbonate from water due to its unique, C-4 like photosynthetic pathway (Holaday & Bowes 1980). *Hydrilla verticillata* apical stem fragments were collected from culture ponds at the Lewisville Aquatic Ecosystem Research Facility, Lewisville, TX.

The pH drift technique described by Allen and Spence (1981) was used to determine the HCO_3^- uptake potential of Texas wild rice. This method involves continuous measurement of pH in a solution bathing actively photosynthesizing plants in a closed system at constant alkalinity (ALK). The method relies on shifts in abundance of the three species of dissolved inorganic carbon (CO_2, HCO_3^-, $CO_3^=$) with pH. In a closed system, plants will continue to photosynthesize until inorganic carbon can no longer be extracted from the bathing solution. When photosynthesis ceases, the final pH of the solution is dependent on the concentration of total dissolved inorganic carbon (C_T) in solution which depends upon the carbon-extracting potential of the plant.

The pH endpoint of these experiments varies with a plant's ability to utilize HCO_3^- and on the alkalinity of the solution. Carbon dioxide obligate plants (those which cannot utilize HCO_3^-) cannot shift the pH of the bathing solution past the point where free CO_2 disappears from solution (pH ca. 9.2 depending

on alkalinity). The CO_2 content of the solution at the pH where photosynthesis ceases is considered the CO_2 compensation point for that species. Removal of CO_2 from the water results in increased pH, but has little impact on C_T, and does not impact ALK.

Other aquatic plants are also able to utilize HCO_3^- as a source of inorganic carbon. In a closed system, these plants will shift the pH well above the threshold where CO_2 disappears. In this case, the final pH of the solution depends on the total C_T pool available as well as the affinity of the plant for HCO_3^-. The HCO_3^- content of the solution at the pH where photosynthesis ceases is considered the HCO_3^- compensation point for that species. The removal of HCO_3^- from solution has a stronger impact on C_T but still does not impact ALK, since the buffering capacity lost by the consumed HCO_3^- is balanced by the production of OH^-. The C_T:ALK ratio at the end of a pH drift experiment is considered a relative measure of a plant's ability to utilize HCO_3^- (Maberly and Spence 1983). The C_T:ALK ratio of CO_2-obligate plants is very close to 1.0, while the ratio of HCO_3^- users is well below 1.0.

Oxygen and pH were continuously measured in a re-circulating closed system. The experimental apparatus consisted of a glass incubation chamber, an acrylic probe block, and a submersible centrifugal pump, all connected by gas tight tubes of butyl rubber (total volume 400 ml) and submerged in a temperature-controlled water bath (20° C). The incubation chamber was a glass cylinder 5 cm in diameter × 30 cm long. A glass tube connector was fused to one end, while a ground-glass fitting with another glass tube connector was fused to the opposite end to allow access to the cylinder for plant tissue insertion and removal. Water was re-circulated at an approximate velocity of 2 cm s^{-1} through the incubation chamber and was sufficient to remove diffusive limitations of photosynthesis. The acrylic sensor mounting block allowed both pH and O_2 sensors to be screwed in so that the electrode tips were continually bathed by the re-circulating solution. The pH electrode (Ross combination electrode) had a sensitivity of 0.01 pH units and was connected to a pH transmitter (Jenco 629). The fast-response, low-consumption Clark-type polarographic oxygen sensor (YSI 5331) was connected to an oxygen meter (Cameron Instrument Corp). The oxygen electrodes were calibrated by submersing the probe in N_2-sparged water and fully aerated water at incubation temperature. pH sensors were calibrated prior to use utilizing pH 7.0 and 10.0 buffers. Outputs from the pH transmitter and the oxygen meter were continuously monitored by a computer equipped with data acquisition software (Strawberry Tree Workbench PC). The sensors were monitored continuously and data recorded every 3 minutes. Calibrations did not drift over the time-scale of the experiments. Alkalinity of the bathing solutions were determined by titration with dilute hydrochloric acid (APHA 1992).

Entire Texas wild rice rosettes consisting of 3–6 leaves (0.2–0.3 gdw) were used in the experiments. Light was provided by specialized fluorescent aquaria lights (6700 K) providing saturating light intensities (500 μmol m^{-2} s^{-1} PAR). The bathing solutions used for the experiments were either San Marcos River water (Alk = 4.5-5.0 meq l^{-1}), a general purpose culture solution (Alk = 0.82 meq l^{-1}; Smart and Barko 1985), or intermediate mixtures of the two. Prior to the start of the experiment, the incubation chambers were flushed with a bathing solution which had been bubbled with a gas mixture composed of 350 ppm CO_2 (atmospheric concentration) and 6% O_2 (approximately 30% atmospheric concentration) in nitrogen. This reduced the O_2 concentration to about 75 μmol l^{-1} while maintaining normal dissolved inorganic carbon and pH levels of the bathing solutions. Lowering the initial O_2 concentration allows photosynthesis to proceed for extended periods within the closed system without potential complications due to O_2 supersaturation which increases photorespiration.

Photosynthesis was measured as the rate of O_2 evolution in the closed system through time. Corrections were made for total volume of the incubation apparatus, and the results normalized to plant dry weight. Experiments were allowed to proceed until photosynthesis stopped and the pH stabilized. This usually consisted of 2 to 3 hour runs, although longer runs up to 12 hours were made to verify that steady state conditions were achieved in the shorter runs.

Concentrations of different carbon species as a function of pH and temperature were estimated from equilibrium constants and formulae in Stumm and Morgan (1981).

RESULTS

Table 1 shows the results of 15 independent pH drift experiments conducted on Texas wild rice and three experiments conducted on *H. verticellata*. Alkalinity was measured prior to the experiment for each bathing solution as was the final pH at the apparent photosynthetic compensation point. Total carbon and CO_2 were estimated based on these data. The C_T:ALK ratio for all experiments was very close to 1.0 and varied between 0.93–0.99, strongly suggesting that this species is a CO_2 obligate plant which is unable to utilize HCO_3^- at a rate sufficient to keep up with photosynthetic needs (see Maberly and Spence 1983). For CO_2-obligates, the CO_2 concentration at the end of the pH drift experiment represents a conservative estimate of the CO_2 compensation point for the species. In this series of experiments, the values varied from 1–13 μmol l^{-1}.

Rates of photosynthesis as a function of pH for two C_T concentrations are shown in Figure 1A. As expected, the rates of photosynthesis at a given pH are higher for the higher C_T solution. Since pH controls the proportion of C_T present as CO_2, the level of CO_2 present in the two solutions was actually very different. However, both data sets show a linear and strong reduction in photosynthesis as the pH of the solution approaches 9.0. In Fig. 1B these data are re-plotted to

Table 1. Summary of pH drift experiments for Texas wild rice and *Hydrilla verticillata* conducted in closed systems including alkalinity (Alk) of bathing solution, final pH, final concentration of total inorganic carbon (C_T), CO_2 remaining at the end of the experiment and the ratio of C_T/Alk.

Run ID	Alkalinity (meq l^{-1})	Final pH	C_T (mmol l^{-1})	CO_2 (µmol l^{-1})	C_T/Alk
Texas wild rice (San Marcos River)					
1	1.24	8.54	1.225	8	0.99
2	1.24	8.66	1.216	6	0.98
3	1.24	8.76	1.207	5	0.97
4	2.00	8.54	1.978	13	0.99
5	2.00	8.72	1.956	8	0.98
6	2.00	9.14	1.876	3	0.94
7	2.16	9.21	2.006	3	0.93
8	2.04	9.16	1.908	3	0.94
9	2.04	9.14	1.914	3	0.94
10	0.82	8.81	0.796	3	0.97
11	0.82	8.72	0.801	4	0.98
12	0.83	9.14	0.778	1	0.93
13	2.45	8.80	2.391	9	0.98
14	2.34	8.63	2.308	13	0.99
15	2.40	9.18	2.254	3	0.94
Hydrilla verticillata (pond culture)					
1	0.85	10.43	0.377	0.01	0.44
2	0.85	10.36	0.414	0.02	0.49
3	0.85	10.23	0.477	0.04	0.56

show the relationship of photosynthesis to actual CO_2 levels. The linear nature of this curve at low CO_2 is characteristic of CO_2-obligate plants and the extrapolated X-axis intercept of 2 µmol l^{-1} provides a reliable estimate of the CO_2-compensation point of photosynthesis for this species (Sand-Jensen 1983).

DISCUSSION

The experimental method used in this study identifies the upper pH limit for positive net photosynthesis. Net photosynthesis by Texas wild rice approached zero as pH approached 8.7 when CO_2 was 0.5% of total dissolved inorganic carbon. Bicarbonate in the bathing solution at pH 8.7 was 97.5% of total dissolved inorganic carbon. At this pH there was abundant HCO_3^- available for plant uptake. If Texas wild rice had the ability to remove HCO_3^- rapidly, positive net photosynthesis would continue after CO_2 was exhausted, driving pH above 9.2. During this study, no photosynthesis occurred above 8.7 suggesting submersed leaves of Texas wild rice are unable to take up HCO_3^-.

Carbon dioxide availability to submersed aquatic plants is a function of the interplay between water current velocity, leaf boundary layer thickness and
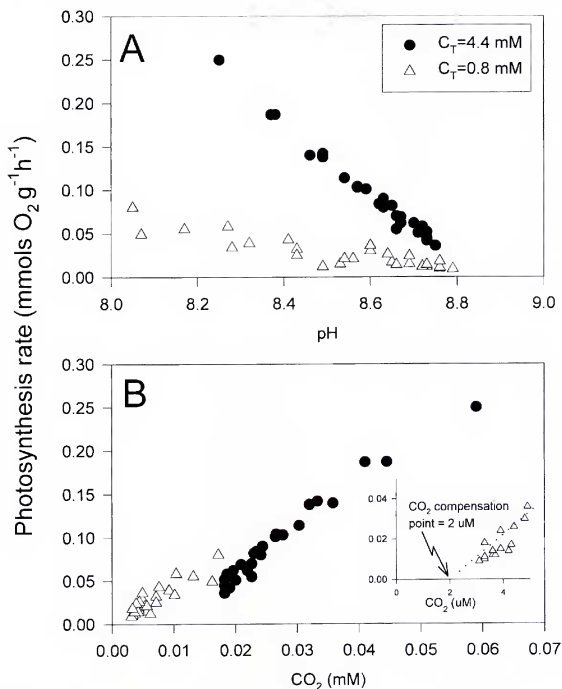
Fig. 1. Apparent net photosynthetic rate by Texas wild rice in a closed water-flow system. A) Plants take up CO_2 for photosynthesis, driving up pH. Photosynthesis ceases when pH reaches approximately 8.7. B) Estimated CO_2 compensation point for Texas wild rice is 2 µmol l^{-1}.

concentration gradient between surrounding water and leaf surface; as flow increases, boundary layer thickness decreases and CO_2 availability increases. Using a closed water-flow system Madsen and Søndergaard (1983) found apparent photosynthesis was stimulated by increasing velocities and a maximum rate was reached at 0.008–0.0012 m s^{-1}. In contrast, Madsen et al. (1993) found

photosynthetic rates decreased with increasing current velocity and suggested that physical stress imposed on plants by agitation or stretching in flowing water was a key factor in the observed response. Ribbon-like submersed leaves of Texas wild rice are adapted for flowing water and can reduce carbon limitation by exploiting the flowing water habitat where boundary layer surrounding leaves and diffusion distances for CO_2 are reduced and leaves are continually bathed with carbon-rich water. In contrast, in slower moving water, photosynthesis by submersed leaves of Texas wild rice are probably carbon limited and few submersed leaves are produced, a greater proportion of biomass is allocated to reproductive parts, and plants are less productive overall (Power 2002). Emergent reproductive culms most likely are not carbon limited because culms obtain CO_2 from the atmosphere where CO_2 is more readily available owing to the higher diffusion rate and current velocity in air relative to water (Madsen & Sand-Jensen 1991; Denny 1993).

Texas wild rice is one of five endangered or threatened species which occur in the San Marcos River. As the result of a lawsuit involving the listed species, a water authority was created by the Texas Legislature to manage the source aquifer for the San Marcos River (Texas Legislature 1993). Flow requirements for Texas wild rice are important because of the legal requirement to manage the source aquifer for human needs while simultaneously protecting aquatic and wildlife habitat and protecting listed species. The potentially conflicting management goals of providing aquifer water for human use while maintaining adequate spring flow for endangered species cannot be attained without information concerning the habitat requirements for the listed species. Numerous surveys have identified flowing water habitat as a requirement for Texas wild rice and experimental studies have consistently shown a positive relationship between current velocity and productivity as well as biomass allocation to submersed plant parts (Power 1996a, 1996b, 2002; Poole & Bowles 1999; Saunders et al 2001). Data from this study provide a physiological explanation for these observations and furthers our understanding of habitat requirements for Texas wild rice.

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